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Isolation and characterization of *Acidobacterium ailaaui* sp. nov., a novel member of *Acidobacteria* subdivision 1, from a geothermally heated Hawaiian microbial mat

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A novel member of *Acidobacteria* was isolated from a microbial mat growing on a geothermally heated dead tree trunk in Hawai'i Volcanoes National Park (HI, USA). The rod-shaped, Gram-negative capsulated cells of strain PMMR2^T were non-motile and catalase and oxidase negative. Growth occurred aerobically from 15 to 55 °C (optimum, 40 °C) and at pH values from 4.5 to 7.0 (optimum, 6.5). A limited range of sugars and organic acids supported growth. However, results of a genomic analysis suggested that various polysaccharides might be hydrolysed as carbon sources, and evidence for pectin degradation was observed in liquid cultures. A genomic analysis also revealed genes for a Group 1f uptake hydrogenase; assays with liquid cultures confirmed hydrogen consumption, including uptake at sub-atmospheric concentrations. Nitrate was not dissimilated to nitrite. Major membrane fatty acids included iso-$C_{15:0}$ and iso-$C_{17:0}$. The G+C content was 57.2mol%. A comparative genome analysis revealed an average nucleotide identity of 72.2 % between PMMR2^T and its nearest cultured phylogenetic neighbour, *Acidobacterium capsulatum* ATCC 51196^T (=JCM 7670^T); analysis of the 16S rRNA gene revealed a 96.8 % sequence identity with *Acidobacterium capsulatum* ATCC 51196^T. These results and other phenotypic differences indicated that strain PMMR2^T represents a novel species in the genus *Acidobacterium*, for which the name *Acidobacterium ailaaui* sp. nov. is proposed. The type strain, PMMR2^T (=DSM 27394^T=LMG 28340^T), is the second formal addition to the genus *Acidobacterium*.

The phylum *Acidobacteria* occurs commonly in soil (Dunbar *et al.*, 1999; Chan *et al.*, 2006; Barns *et al.*, 2007; Lee & Cho, 2009; Jones *et al.*, 2009; Kielak *et al.*, 2009; Davis *et al.*, 2011). Based on results from 16S rRNA gene clone libraries and 'next-gen' sequencing, members of *Acidobacteria* represent 20–80 % of the richness of soil microbial communities (Dunbar *et al.*, 1999; Janssen, 2006; Chan *et al.*, 2006). *Acidobacteria* have also been reported in fresh water and marine systems, including hydrothermal vents in the deep sea (Fukunaga *et al.*, 2008; Zeng *et al.*, 2011; Izumi *et al.*, 2012; Foesel *et al.*, 2014).

Since the first description of *Acidobacterium capsulatum* JCM 7670^T (Kishimoto *et al.*, 1991), only a few tens of isolates have been validly published. Molecular ecological studies of 16S rRNA gene sequences remain the primary source of information about *Acidobacteria*. Analyses of 16S rRNA gene sequence data have indicated that 26 distinct subdivisions comprise *Acidobacteria* and that subgroup distribution varies with habitat type (Jones *et al.*, 2009). Members of subdivisions 1, 3, 4 and 6 typically dominate soil, while subdivisions 7 and 8 and 10, 22 and 23 dominate *Acidobacteria* in a cave mat community and a Bering Sea sediment, respectively (Zimmermann *et al.*, 2005; Janssen, 2006; Meisinger *et al.*, 2007; Jones *et al.*, 2009; Zeng *et al.*, 2011). In addition, thermophiles from subdivisions 4, 10 and 23 have been reported in hot springs and a hydrothermal vent (Izumi *et al.*, 2012; Losey *et al.*, 2013; Crowe *et al.*, 2014).

To date, most of the isolates have been obtained from subdivision 1 (Kishimoto *et al.*, 1991; Eichorst *et al.*, 2007; Koch *et al.*, 2008; Pankratov & Dedysh, 2010; Männistö *et al.*, 2012; Okamura *et al.*, 2011; Dedysh *et al.*, 2012; Kulichevskaya *et al.*, 2012; Pankratov *et al.*, 2012; Rawat

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One supplementary figure is available with the online Supplementary Material.

et al., 2012), with cultured representatives reported for 3, 4, 8, 10 and 23 (Kulichevskaya *et al.*, 2010; Izumi *et al.*, 2012; Foesel *et al.*, 2013; Losey *et al.*, 2013; Huber *et al.*, 2014). Notable isolates include *Acanthopleuribacter pedis* (subdivision 8, from a chiton; Fukunaga *et al.*, 2008), *Geothrix fermentans* (subdivision 8, a ferric iron reducer; Coates *et al.*, 1999) and '*Chloracidobacterium thermophilum*' (subdivision 4, an anoxygenic phototroph; Tank & Bryant, 2015). We report here the isolation of a novel hydrogen-oxidizing thermotolerant member of the genus *Acidobacterium*. Strain PMM2^T is acid tolerant, chemoorganotrophic, aerobic and an important constituent of unusual microbial biofilms growing on the surfaces of dead, geothermally heated tree trunks.

Strain PMMR2^T was isolated from geothermally heated microbial mats collected from the Puhimau geothermal area in Hawai'i Volcanoes National Park. The site originally supported a *Metrosideros polymorpha* forest, but an underground magma intrusion in the 1930s killed the vegetation, leaving behind dead tree stumps that now act as steam vents (Smith, 1981). At the time of sampling, many of the tree stumps supported distinct, reddish-brown mats (pH 3–4) dominated by *Acidobacteria* (subdivisions 1 and 3) and *Ktedonobacteria* (King & King, 2014a). Previous studies isolated representative novel CO-oxidizing *Ktedonobacteria* and *Chloroflexi* from these mats (King & King, 2014b). In an effort to isolate representative *Acidobacteria*, mat samples were collected and transferred to Whirl-Pak bags (Nasco) that were shipped to Louisiana State University (Baton Rouge, LA, USA) for further processing.

Mat subsamples were homogenized and then diluted serially from 10^{-2} to 10^{-8} in 1/10 R2A at pH 4 with 0.2 % pectin used as a buffer. Dilutions were incubated at 30 °C for 3 weeks. The highest dilution with growth was streaked onto 1/10 R2A (pH 4) agar plates and incubated at 30 °C. Individual colonies were selected and inoculated into 1/10 R2A broth (pH 4). Enrichments were re-plated as needed to obtain a pure culture designated as strain PMMR2^T. Since the isolate obtained during this process appeared capable of using pectin in liquid culture and raising the medium pH to 5.5, subsequent media were buffered with 10 mM MES at pH 5.5.

Genomic DNA was extracted from a liquid culture of strain PMMR2^T using a MO BIO Ultraclean Microbial DNA extraction kit (MO BIO). Purified DNA was sequenced using Illumina Hiseq and PacBio platforms by the Department of Energy Joint Genome Institute. These platforms generated reads of 270 bp and 10 000 bp, respectively; reads were assembled into a single contig using the Hierarchical Genome Assembly Process (HGAP v. 2.0.1; Chin *et al.*, 2013). Genes were called and annotated using Prodigal (v. 2.5; Hyatt *et al.*, 2010) as part of the Department of Energy Joint Genome Institute Integrated Microbial Genomes system's Microbial Annotation Pipeline. This whole genome shotgun-sequencing project has been deposited as GenBank accession JIAL00000000.

The PMMR2^T genome was composed of 3 686 523 bp, which was similar to the 4 127 356 bp genome size of its nearest phylogenetic neighbour, *Acidobacterium capsulatum* ATCC 51196^T, but quite a bit smaller than the genomes of '*Silvibacterium bohemicum*' S15^T (Lladó *et al.*, 2016) and other *Acidobacteria* in the Integrated Microbial Genomes database. Genomes of strain PMMR2^T and *Acidobacterium capsulatum* ATCC 51196^T harboured 3184 and 3425 genes, respectively, with 3131 and 3377 protein-coding genes each. In strain PMMR2^T, 867 genes were identified as coding for enzymes, and 198 were identified as transporters. A total of 906 genes were associated with KEGG pathways, 1980 with COGs, and 769 with MetaCyc pathways. An average nucleotide identity of 72.2 % was derived from a comparison of the genomes of strain PMMR2^T and *Acidobacterium capsulatum* ATCC 51196^T. This is well below the recommended cut-off of 95.0–96.5 % for species differentiation (Konstantinidis & Tiedje, 2005).

Genomic DNA extracts were also used to amplify the PMMR2^T 16S rRNA gene by PCR with universal bacterial primers 27F and 1492R (Lane, 1991). The amplicon was cleaned using an UltraClean PCR Clean-up Kit (MO BIO Laboratories). Purified DNA was sequenced with an ABI 3130XL Genetic Analyzer (Applied Biosystems) at the Louisiana State University Genomics Facility. The bidirectional sequence reads were assembled and edited using Sequencer 4.8 (Sequencher sequence analysis software, Gene Codes). The 16S rRNA gene sequence (1258 bp) was aligned with sequences from other *Acidobacteria* using the SINA alignment service (Pruesse *et al.*, 2012). Phylogenetic relationships among the sequences were determined using a maximum-likelihood analysis in MEGA v. 6.06 (Tamura *et al.*, 2013). PMMR2^T formed a cluster in the subdivision 1 *Acidobacteria* with its nearest phylogenetic neighbour, *Acidobacterium capsulatum* ATCC 51196^T, and was clearly distinct from all other genera in the subdivision (Fig. 1). EzTaxon (http://www.ezbiocloud.net/) was used to determine identities for nearest phylogenetic neighbours (Kim *et al.*, 2012). Based on an EzTaxon analysis, a 16S rRNA gene sequence identity of 96.8 % was obtained from a comparison of strain PMMR2^T and *Acidobacterium capsulatum* ATCC 51196^T. This is well below the accepted cut-off for 16S rRNA gene-based species differentiation of 98.7 % (Yarza *et al.*, 2014).

A French press was used to lyse cells to obtain DNA for mol% G+C determinations. DNA was hydrolysed (Mesbah *et al.*, 1989), and the resulting nucleotide bases quantified by the Deutsche Sammlung von Mikroorganismen und Zellkulturen on an HPLC system with a VYDAC 201SP54, C_{18}, 5 µm (250×4.6 mm) column (Shimadzu) following standard methods (Tamaoka & Komagata, 1984). The G+C content for strain PMMR2^T was 57.2 mol% (Table 1), which was somewhat higher than the value of 56.5 mol% obtained from its genome sequence. G+C content values of 59.9–60.8 and 58.2 mol% have been reported for *Acidobacterium capsulatum* JCM 7670 and '*Silvibacterium*

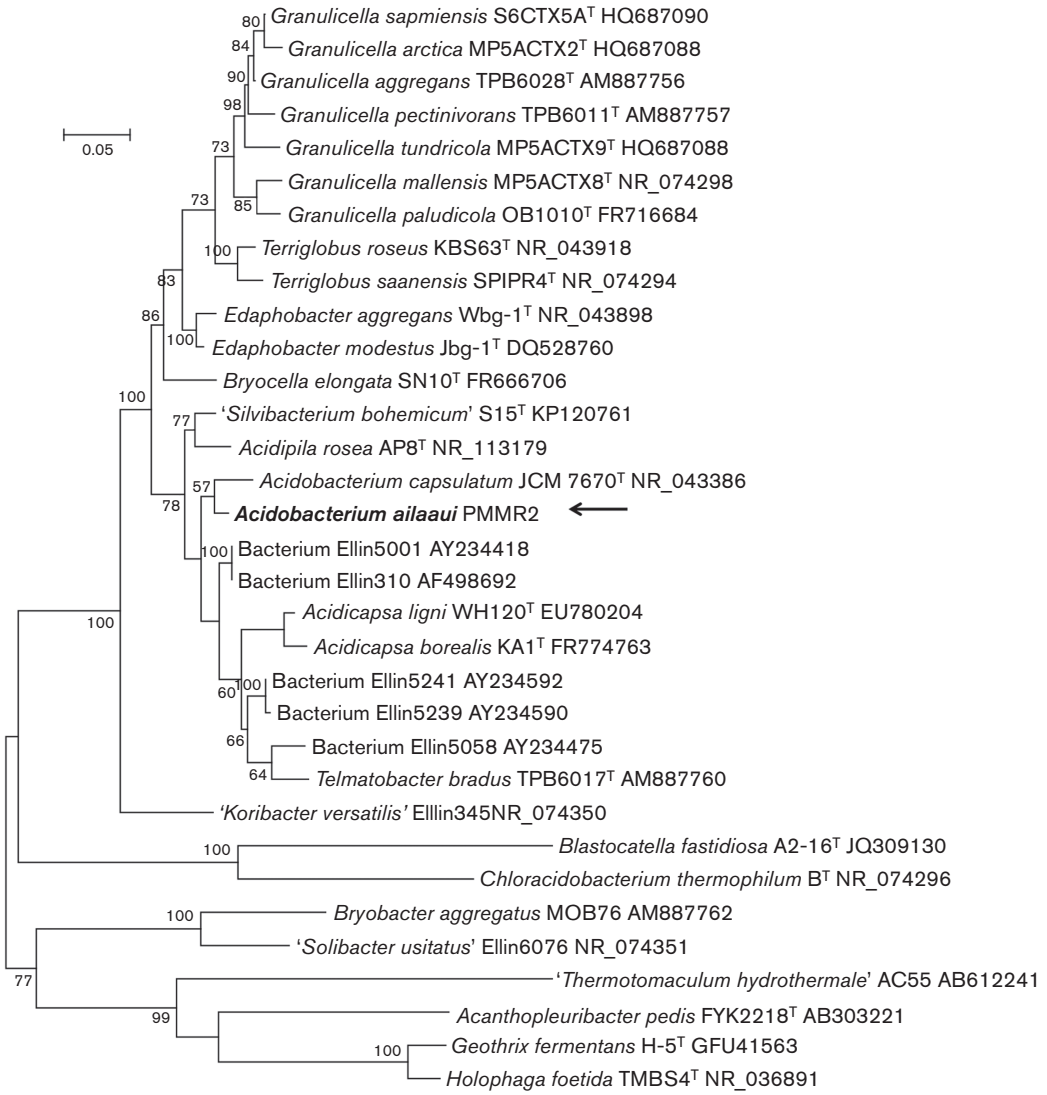

Fig. 1. Phylogeny of *Acidobacterium ailaaui* PMMR2^T based on a SINA alignment of 16S rRNA gene sequences and maximum-likelihood algorithm with a general time-reversible model. Bootstrap percentages (100 replicates) are indicated below the branches. Evolutionary rate differences among sites were modelled with a discrete gamma distribution [5 categories (+G, parameter=0.4431)]; 39.403 % (+I) of sites were invariable. A total of 1244 positions comprised the final dataset. Arrow denotes PMMR2^T.

bohemicum' S15^T, respectively (Table 1; Kishimoto *et al.*, 1991; Lladó *et al.*, 2016).

Cellular fatty acid composition was determined commercially by the Deutsche Sammlung von Mikroorganismen und Zellkulturen. Membrane lipid fatty acids were extracted from cells grown on solid media (1/10 R2A); fatty acids were methylated as described by Miller (1982) and Kuykendall *et al.* (1988). Fatty acid methyl ester mixtures were analysed using the Sherlock Microbial Identification System (MIDI, Microbial ID). This system utilized an Agilent model 6890N gas chromatograph fitted with a 5 % phenyl-methyl silicone capillary column (0.2 mm×25 m), a flame ionization detector and an Agilent model 7683A automatic sampler (Agilent Technologies). Fatty acid peaks were identified using the Microbial Identification System Standard Software (Microbial ID).

iso-$C_{15:0}$ dominated the membrane lipid fatty acids of strain PMMR2^T (61.5 %) as has been reported for other members of *Acidobacteria* subdivision 1 (Table 2). PMMR2^T shared a number of additional fatty acids with members of subdivision 1, including $C_{15:0}$, $C_{16:0}$, iso-$C_{17:0}$ and $C_{18:0}$. $C_{16:1}\omega7c$ occurred in all subdivision 1 isolates and was represented in PMMR2^T by summed feature 3 (Table 2). Membranes of strain PMMR2^T appeared

Table 1. Phenotypic characteristics of novel strain PMMR2^T and related type strains of subdivision 1 of *Acidobacteria*

Strains: 1, PMMR2^T; 2, *Acidobacterium capsulatum* JCM 7670^T; 3, '*Silvibacter bohemicum*' S15^T; 4, *Acidicapsa borealis* DSM 23886^T; 5, *Telmatobacter bradus* DSM 23630^T; 6, *Edaphobacter modestus* Jbg-1^T; 7, *Granulicella paludicola* OB1010^T; 8, '*Terracidiphilus gabretensis*'; 9, *Bryocella elongata* SN10^T. Colony colours: W, white; LB, light beige; B, beige; PO/O, pale orange/orange; P, pink; LP, light pink; T, transparent; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9
Colony colour	W/LB	PO/O	W	PP	W/B	B	P	T	LP
Cell size (μm)									
Length	1.5±0.2	1.1–2.3	0.9–1.1	1–3	2.0–10.0	1.0–1.8	1.5–3.5	0.6–1.2	1.7–4.0
Width	0.8±0.1	0.3–0.8	0.3–0.5	0.6–0.9	0.4–0.6	0.5–0.7	0.4–0.6	0.5–0.8	0.7–1.0
G+C content (mol%)	57.2	59.9–60.8	58.2	54.1	57.6	55.8	57.4	57.3	60.7
Temperature (°C)									
Optimum	40	30	22–24	22–28	20–28	30	18–22	20–24	20–24
Range	30–55	20–37	20–28	10–33	4–35	15–30	2–33	12–30	6–32
pH:									
Optimum	6.5	5	4–5	5.0–5.5	4.5–5.0	5.5	4.2	4–5	4.7–5.2
Range	4.5–7	3.0–6.0	3–6	3.5–7.3	3.0–7.5	4.5–7.0	3.0–7.5	3–6	3.2–6.6
Anaerobic growth	+	weak	−	−	+	ND	ND	−	−
Microaerophilic growth	+	+	−	ND	+	ND	ND	−	−
Catalase	−	+	weak	+	weak	+	+	−	+
Oxidase	−	−	−	−	−	+	+	−	−
Growth with:									
1 % NaCl	−	ND	ND	+	−	ND	+	−	+
Hydrolysis of:									
Gelatin	+	−	ND	−	ND	ND	ND	ND	ND
Aesculin	+	+	ND	ND	ND	ND	ND	ND	ND
Growth substrates									
Malic acid	−	ND	+	−	−	−	−	+	−
Glucuronic acid	+	+	ND	+	+	ND	ND	ND	−
Alanine	−	−	+	ND	ND	−	ND	ND	ND
Glycerol	+	−	+	−	ND	−	ND	+	−
Glucose	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	−	+	+	+
Lactose	+	+	ND	+	+	+	+	ND	+
Sucrose	+	ND	+	+	+	ND	+	+	+
Ribose	−	ND	ND	+	−	ND	−	ND	ND
Mannose	+	+	+	+	+	ND	+	+	+
Arabinose	+	+	+	+	+	+	−	ND	ND
Xylose	+	+	+	+	+	+	ND	+	+
Fructose	+	+	+	+	+	+	+	ND	+

uniquely to contain anteiso-$C_{15:1}$ and iso-$C_{17:1}\omega 9c$ (9.3 %; Table 2). In addition, iso-$C_{17:0}$ occurred in strain PMMR2^T at relatively high amounts compared to other isolates (12.3 % versus 1.4–3.8 %, Table 2). Strain PMMR2^T lacked $C_{18:1}\omega 9c$, a feature shared by several other strains (Table 2). Relative to its closest phylogenetic neighbour, *Acidobacterium capsulatum* JCM 7670^T, multiple differences in fatty acid composition were apparent, consistent with species level differentiation.

Colony and cell characteristics were determined after growth for 6 d at 40 °C on R2A medium (pH 5.5). Cell morphology and dimensions were determined using a Zeiss Axioscope and an AxioCam MR digital camera. Gram staining was performed using standard methods. Strain PMMR2^T formed light beige, round, smooth, opaque colonies. Cells occurred singly or in pairs; division occurred by binary fission. Cells were Gram-negative, aerobic, not spore forming, rod shaped (1.5±0.2 μm×0.8±0.1 μm), non-motile and capsulated. The lack of motility was notable, since the genome of PMMR2^T contains 34 genes for flagellum synthesis, indicating that motility might occur under conditions different from those used in this study.

Acidobacterium capsulatum JCM 7670^T has been reported as motile and capsulated; its motility differs from that of PMMR2^T, but that might not be a significant diagnostic trait. '*Silvibacterium bohemicum*' S15^T, another close

Table 2. Fatty acid composition for novel strain PMMR2 and related type strains of subdivision 1 of *Acidobacteria*

Strains: 1, PMMR2^T; 2, *Acidobacterium capsulatum* JCM 7670^T; 3, *Granulicella arctica* MP5ACTX2^T; 4, *Acidicapsa borealis* KA1^T; 5, '*Silvibacter bohemicum*' S15^T, TR, Trace.

Fatty acid	1	2	3	4	5
$C_{15:0}$	1.8	1	TR	1.9	8.3
iso-$C_{15:0}$	61.53	55.8–65.0	55.8	55.4	73.7
anteiso-$C_{15:0}$	–	–	–	0.3	–
$C_{16:0}$	2.3	4.0–4.3	5.3	5.9	–
iso-$C_{16:0}$	–	–	–	–	0.4
$C_{17:0}$	3.4	1.4–1.9	–	–	–
iso-$C_{17:0}$	12.3	2.2–3.0	1.4	3.8	3.2
anteiso-$C_{17:0}$	0.5	–	–	2.0	–
10-methyl $C_{17:0}$	0.8	–	0.7	–	–
$C_{18:0}$	2.5	13.3–13.8	–	1.6	2.7
anteiso-$C_{18:0}$	–	–	–	12.5	–
12-methyl $C_{18:0}$	–	–	57.3	–	–
$C_{19:0}$	–	–	2.6	6.6	–
anteiso-A $C_{15:1}$	0.2	–	–	–	–
$C_{15:1}\omega6c$	0.3	–	0.5	–	–
$C_{16:1}\omega7c$	–	3.5–4.0	31.7	0.8	8.6
$C_{17:1}\omega8c$	0.6	2.3–3.9	–	–	0.6
iso-$C_{17:1}$	–	–	0.6	–	–
iso-$C_{17:1}\omega8c$	–	–	–	25.5	–
iso-$C_{17:1}\omega9c$	9.3	–	–	–	–
$C_{18:1}\omega9c$	–	12.4–15.2	–	1.9	2.2
Summed feature 1 (iso-$C_{15:1}$/$C_{13:0}$ 3-OH)	0.6	–	0.2	–	–
Summed feature 3 ($C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH)	3.0	–	–	–	–
Total	96.8	96.6–111.1	99.6	99.1	99.7

relative of PMMR2^T, has been described as uncapsulated, which differs from PMMR2^T (Lladó *et al.*, 2016) However, like PMMR2^T, '*Silvibacterium bohemicum*' S15^T is non-motile yet harbours a full complement of the genes required for flagellum synthesis (Lladó *et al.*, 2016). The conditions under which these genes are expressed are currently unknown.

Growth assays under sub-oxic (0.2 % oxygen) and anoxic conditions (100 % N_2) were performed in sealed 60 cm^3 serum bottles containing 5 ml R2A medium at pH 5.5. Changes in absorbance (A_{600}) over 7 days were used to assess growth relative to oxic incubations. Limited growth was observed for sub-oxic and anoxic incubations (ΔA_{600}=0.11 and 0.09, respectively) compared to the oxic treatments (ΔA_{600}=0.57), possibly indicating a capacity for fermentation. Weak growth under these conditions is consistent with traits of other subdivision 1 *Acidobacteria* (Kishimoto *et al.*, 1991; Pankratov *et al.*, 2012; Lladó *et al.*, 2016).

The capacity of strain PMMR2^T to utilize different substrates for growth was assessed with a basal medium supplemented with 0.01 % yeast extract (Meyer & Schlegel, 1978). Substrates were added at 25 mM final concentrations, and the medium was buffered using 10 mM MES to pH 5.5. Growth occurred on the following sole carbon sources: arabinose, fructose, galactose, glucose, glucuronic acid, gluconic acid, glycerol, lactose, mannose, proline, succinate, sucrose and xylose. Strain PMMR2^T did not grow with the following sole carbon sources: acetate, acetone, alanine, aspartate, dimethylamine, ethanol, fumarate, glycerate, glycine, glycine betaine, isopropanol, lactate, malate, malonate, methanol, methylamine, propionate, pyruvate, ribose, serine, tartrate, trimethylamine or valine. Strain PMMR2^T used both ammonium and nitrate as sole nitrogen sources; the absence of nitrogenase genes in the PMMR2^T genome indicates that diazotrophy does not occur. *Acidobacterium capsulatum* JCM 7670^T also grows on a limited range of sugars and simple organics but was not reported to use glycerol (Kishimoto *et al.*, 1991). Substrate utilization by '*Silvibacterium bohemicum*' S15^T was more extensive and included growth on ethanol, methanol and tartrate that were not used by PMMR2^T.

Substrate utilization profiles were also assessed with a Biolog GN2 microplate following the manufacturer's recommendations (Biolog). Positive reactions included fructose, fucose, galactose, gentibiose, glucose, lactose, lactulose, maltose, mannose, melibiose, sucrose, trehalose and turanose. Responses to the remaining 82 substrates in the GN2

system were negative. These results were consistent with assays of sole carbon sources and differ markedly from the 49 positive substrates documented for '*Silvibacterium bohemicum*' S15^T (Lladó *et al.*, 2016). Differences in substrate utilization between strain PMMR2^T and '*Silvibacterium bohemicum*' S15^T no doubt reflect large differences in genome size, 3.7 Mb versus 6.4 Mb, respectively.

In addition to monomeric substrates, PMMR2^T might also be able to use a variety of polysaccharides. PMMR2^T was able to hydrolyse pectin, and submission of inferred protein sequences from the genome to the dbCAN database for annotating carbohydrate active enzymes (http://csbl.bmb.uga.edu/dbCAN/) yielded annotations for 14 glucosidases, 11 galactosidases, 9 xylosidases, 4 mannosidases and 3 α-fucosidases. However, enzymes likely active in cellulose degradation were not observed, and cellobiose was not oxidised in assays using a Biolog GN2 microplate.

The presence of Group 1f hydrogenase genes (e.g. WP_026441619.1; *sensu* Piché-Choquette *et al.*, 2016) in the PMMR2^T genome suggested that hydrogen uptake might be possible. Hydrogen uptake was assessed using stationary phase cells grown in R2A medium at pH 5.5. Ten millilitre volumes of a culture with an absorbance (600 nm) of 0.9 were incubated at 40 °C with shaking (100 r.p.m.) in triplicate 60 ml serum bottles sealed with neoprene stoppers. Hydrogen was added to serum bottle headspaces at final concentrations of about 10 p.p.m., and uptake was monitored by removing 100 µl headspace subsamples at intervals for gas chromatographic analysis using a Peak Performer 2 reduced gas detector (Peak Laboratories). Hydrogen concentrations declined via a first-order process to concentrations well below ambient levels [about 0.5–0.6 p.p.m., Fig. S1 (available in the online Supplementary Material)]. Hydrogen uptake might therefore supplement heterotrophic metabolism or support dormancy in PMMR2T as has been suggested for other *Acidobacteria* (Greening *et al.*, 2015a, b, 2016). However, it is noteworthy that PMMR2T contains a Group 1f hydrogenase, while Group 1h hydrogenases have been implicated in high-affinity hydrogen uptake by soil (Constant *et al.*, 2011; Greening *et al.*, 2015b; Piché-Choquette *et al.*, 2016). Results presented here suggest that PMMR2^T and other bacteria with Group 1f might also contribute to high affinity hydrogen uptake, but additional studies will be needed to evaluate this possibility.

The temperature optimum of strain PMMR2^T was assessed using R2A (pH 5.5) as a growth medium and temperatures from 15 to 60 °C. Cells grew optimally at 40 °C with a range of 15–55 °C. The optimal pH for growth was determined using R2A buffered from pH 4.0 to 8.5 and incubated at 40 °C. Buffering reagents were as follows: 0.1 M solutions of pivalic acid (pH 4.0–4.5), 2-(*N*-morpholino)ethanesulfonic acid (pH 5.0–6.5) and Tris (pH 7.5–8.5). PMMR2^T grew optimally at pH 6.5 and over a range from pH 4.5 to 7.0. Sodium chloride inhibited growth at concentrations $\geq$1 % as determined by plating PMMR2^T on R2A medium (pH 5.5) with NaCl levels from 0 to 4 %. PMMR2^T grew over a wider temperature range and with higher temperature optimum than *Acidobacterium capsulatum* JCM 7670^T and '*Silvibacterium bohemicum*' S15^T; it also grew with a higher pH optimum but not at pH values as low as for *Acidobacterium capsulatum*JCM 7670^T and '*Silvibacterium bohemicum*' S15^T (Kishimoto *et al.*, 1991; Lladó *et al.*, 2016).

Catalase activity was determined by mixing a colony into a 3 % hydrogen peroxide solution. No bubbles were observed within 5 min, indicating a negative reaction. However, two catalase genes have been observed in the PMMR2^T genome, which suggests that catalase activity might be expressed under some conditions, e.g. reactive oxygen stresses *in situ*. Positive and weakly positive catalase reactions have been reported for *Acidobacterium capsulatum*JCM 7670^T and '*Silvibacterium bohemicum*' S15^T, respectively, with positive reactions in general for other subdivision 1 isolates (Kishimoto *et al.*, 1991; Lladó *et al.*, 2016). The oxidase reaction was also negative for PMMR2^T, consistent with reports for *Acidobacterium capsulatum*JCM 7670^T, '*Silvibacterium bohemicum*' S15^T and other members of subdivision 1 (Kishimoto *et al.*, 1991; Lladó *et al.*, 2016). Other enzyme activities and substrate utilization patterns were analysed with API 20 NE test strips (bioMérieux). All reactions were negative with the exception of urease and hydrolysis of aesculin and gelatin. In contrast, *Acidobacterium capsulatum* JCM 7670^T has been reported to express β-galactosidase and not hydrolyse gelatin or produce urease (Kishimoto *et al.*, 1991).

Distinct differences between PMMR2^T and *Acidobacterium capsulatum* JCM 7670^T occur for 16S rRNA gene sequences, genomic sequences (based on average nucleotide identity), substrate utilization, membrane fatty acid composition and a variety of phenotypic and biochemical traits indicate. These differences indicate that PMMR2^T is a novel thermotolerant, heterotrophic and hydrogen-oxidizing member of the genus *Acidobacterium*, for which the name *Acidobacterium ailaaui* sp. nov. is proposed.

Description of *Acidobacterium ailaaui* sp. nov.

Acidobacterium ailaaui (ai.la.au'i. N.L. gen. n. *ailaaui* of the Hawaiian forest-eating fire god Ailaau).

Cells are Gram-negative, aerobic, not spore forming, capsulated, rod shaped (1.5±0.2 µm×0.8±0.1 µm) and non-motile at 40 °C and may occur singly or in pairs of two. When grown on R2A (pH 5.5) plates, colonies are circular, smooth, mucoid and light beige. Catalase and oxidase negative. Cells grow between pH 4.5 and 7.0, with an optimum of 6.5. Growth occurs between 15 and 55 °C with an optimum of 40 °C. Sodium chloride inhibits growth at 1 %. Uses the following sole carbon sources for growth: arabinose, fructose, galactose, glucose, glucuronic acid, gluconic acid, glycerol, lactose, mannose, proline, succinate, sucrose and xylose. Does not grow with the following sole carbon

sources: acetate, acetone, alanine, aspartate, dimethylamine, ethanol, fumarate, glycerate, glycine, glycine betaine, isopropanol, lactate, malate, malonate, methanol, methylamine, propionate, pyruvate, ribose, serine, tartrate, trimethylamine or valine. Uses ammonium and nitrate as sole sources of cellular nitrogen. Oxidizes molecular hydrogen. Does not reduce nitrate to nitrite. Urease positive, hydrolyses gelatin and aesculin. Negative for indole production, glucose fermentation, arginine dihydrolase, β-glucoside and β-galactosidase. Major cellular fatty acids include iso-$C_{15:0}$, iso-$C_{17:0}$ and iso-$C_{17:1}\omega 9c$.

The type strain is PMMR2^T (=DSM 27394^T=LMG 28340^T), isolated from a geothermally heated microbial mat at the Puhimau geothermal area (HI, USA). The DNA G+C contents of the type strain are 52.7 mol% (by HPLC) and 56.5 mol% (by genome sequence).

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